nicotinic acetylcholine receptor and [hybridizes under conditions of high stringency to a sequence of nucleotides in the coding sequence of] has substantial sequence homology to the alpha3-encoding DNA in the plasmid HnACHr$\alpha$3 deposited under ATCC Accession No. 68278.

E4 cont.

75. (Amended) Isolated nucleic acid, comprising[ that includes] a sequence of nucleotides [encoding] that encodes [an] a beta2 subunit of a human nicotinic acetylcholine receptor [and hybridizes under conditions of high stringency to a sequence of nucleotides in the coding sequence of the plasmid HnACHr$\beta$2 deposited under ATCC Accession No. 68279] that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 9.

**REMARKS**

A Petition for extension of time for four months and a check for the requisite fee for the extension, additional claims and this submission under Rule 129(a) accompany this response. Any fees, including those for the extension of time, any additional claims, and/or this submission, that are due may be charged to Deposit Account No. 02-4070.

An unexecuted DECLARATION under 37 C.F.R. §1.132 is attached hereto; the executed original will be submitted upon receipt. This DECLARATION is a reexecuted version of the DECLARATION that was submitted in the parent application U.S. application Serial No. 08/504,455, which is now U.S. Patent No. 5,369,028. Articles, Chini et al. (1992) Proc. Nat'l. Acad. Sci. 89: 1572 and Wada et al. (1989) J. Comp. Neurol. 284: 314-335, to which this DECLARATION refers also accompany this response.

Claims 53-63, 66-68 and 70-96 are presently pending in this application. Claims 54-61, 68 and 73-75 have been amended and claims 76-90 have been added in order to more particularly point out and distinctly claim the subject matter that applicant regards as the invention. The amendments and new claims primarily change the form, not the substance, of the claimed subject

matter. The new claims set forth embodiments described in the original claims. Hence no new matter has been added.

**CORRECTION OF THE FILING RECEIPT AND FILING DATE OF THE APPLICATION AS ENTERED INTO THE PALM SYSTEM**

It is respectfully submitted that the filing date of the above-captioned application was incorrectly entered into the PALM system as April 3, 1991. April 3, 1991 is the International filing date of this application. The 35 U.S.C. §102(e) date of this application should be the date of completion of the requirements for entering the National Stage in the U.S., which November 30, 1992.

Attention is directed to the Decision on Petition Under 37 C.F.R. §1.181, dated February 22, 1994, particularly page 2, which indicates under the heading "Conclusion" that this application will be given a date of 30 November 1992 under 35 U.S.C. §371(c) and 102(e).

Applicant, therefore, respectfully requests a corrected filing receipt that reflects the national stage filing date.

**THE OBJECTION TO CLAIMS 58 and 68**

Claims 58 and 68 are objected to as reciting improper Markush language. It is respectfully submitted that, although use of Markush language is not strictly required, particularly when the number of elements is limited, the claims have been amended as requested. Therefore, the grounds for this rejection are rendered moot.

**THE OBJECTION TO THE SPECIFICATION AND REJECTION OF CLAIMS 53-72 UNDER 35 U.S.C. §112, FIRST PARAGRAPH**

The specification is objected to and claims 53-72 are rejected under 35 U.S.C. §112, first paragraph, as failing to provide an enabling disclosure for the production of substantially pure human neuronal nicotinic receptors.

The Examiner indicates in the Advisory Action, mailed November 15, 1995, that this rejection has been reconsidered and would have been

withdrawn upon entry of the Amendment After Final, mailed October 25, 1995. Consequently, rather than belabor this issue, arguments with respect to this rejection similar to those in the unentered amendment are set forth below. This is done in reliance upon the Examiner's statement in the Advisory Action.

As stated in the Amendment After Final, the claimed subunits readily could have been produced using any of numerous methods known to those of skill in this art at the time of filing this application. For example, as described in the application the nucleic acids can be introduced into and expressed in a suitable host cell. The specification is not required to expressly teach that which is well known to those of skill in the art; such knowledge is implicitly part of the specification. See, Spectra-Physics, Inc. v. Coherent, Inc. 827 F.2d 1524, 3 USPQ2d 1737 (Fed. Cir. 1987).

In this instance, many host cells and the use thereof to express heterologous proteins were well known and widely used by molecular and cellular biologists at the time of filing this application. In addition, the instant specification describes various suitable host cells. For example, at page 14, line 8, the specification teaches:

> Invention DNA sequences can be transformed into a variety of host cells. Eukaryotic cells such as yeast or mammalian cells are presently preferred. A variety of suitable host mammalian cells, having desirable growth and handling properties, are readily available to those of skill in the art. Especially preferred for such purpose are human, rat or mouse cells.
>
> Similarly, a variety of suitable yeast cells are readily available to host cells for the invention sequences. Especially preferred are yeast selected from Pichia pastoris, Saccharomyces cerevisiae, Candida tropicalis, Hansenula polymorpha, and the like.
>
> Alternatively, the invention DNA sequences can be translated into RNA, which can then be transfected into amphibian cells for transcription into protein. Suitable amphibian cells include Xenopus oocytes.

Also, introduction of DNA encoding nicotinic acetylcholine receptors and expression thereof in host cells is exemplified in the application. Example 1 of the specification teaches preparation of an expression vector for expressing the human nicotinic acetylcholine receptors; and Example 2 teaches preparation of cell lines that express rat $\alpha$ and $\beta$ subunits produced by cotransfecting a plasmid comprised of an $\alpha$-subunit-encoding sequence, a plasmid comprised of a $\beta$-subunit-encoding sequence, and a plasmid comprised of either the wild-type or crippled TK gene. Example 2 indicates that the vectors of Example 1 can be used in the same way. Example 4 shows that functional nNAChRs are expressed by mammalian cells transfected with DNA encoding an $\alpha$-subunit and a $\beta$-subunit of the nNAChR. Thus, the specification teaches methods for expression of such receptors.

Finally to evidence that those of skill in this art could recover nicotinic acetylcholine receptors from host cells that express recombinant or heterologous nicotinic acetylcholine receptor, attention is directed to the reference Claudio et al. (1987) Science 238:688-694, which is of record in this application.

**THE REJECTION OF CLAIMS 53-63, 66-68 and 70-75 UNDER 35 U.S.C. §112, FIRST PARAGRAPH**

Claims 53-63, 66-68 and 70-75 are rejected under 35 U.S.C. §112, first paragraph, because the disclosure is allegedly only enabling for DNA encoding a neuronal nicotinic acetylcholine receptor encoded by any of the deposited plasmids. In particular, it is urged that one of skill in the art would have to resort to undue experimentation to rationally design a functional receptor subunit having other than a natural amino acid sequence. The Examiner urges that disclosure of only partial sequences is "clearly insufficient" to support claims to DNA fragments that hybridize to the disclosed DNA under high stringency conditions; and that Applicant's claim limitations are "directly analogous to those of (invalidated) claim 7 of U.S. Patent Number 4,703,008."

This rejection is respectfully traversed insofar as it applies to any of the presently pending claims. It is noted that presently pending claims 79-85, 87 and 96 are not within the purview of this rejection.

**Relevant law**

In order to satisfy the enablement requirement of 35 U.S.C §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409. This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything within the scope of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973). Rather, the requirements of §112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocchi et al., 469 USPQ 367 (CCPA 1971), emphasis added.

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee is not only entitled to narrow claims, particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

**A. The instant claims are not analogous to claim 7 in U.S. Patent No. 4,703,008, which was deemed invalid under 35 U.S.C. §112, first paragraph, as not being enabled by the specification**

First, it is noted that enablement is determined by reference to the teachings in the specification and the knowledge of those of skill in the art at the time of filing (such knowledge is presumed to be part of the application disclosure). Thus, a finding that a claim is of analogous scope to one deemed non-enabled in one case, has no relevance to the case at issue, since enablement is a function of the teachings in the specification. As discussed below (B), the instant specification teaches how to make and use what is claimed without undue experimentation.

Assuming, arguendo, that such determination is relevant, it is respectfully submitted that the instant claims are not analogous to claim 7 of U.S. Patent

No. 4,703,008, but rather are more analogous to claim 2, which was deemed valid.

Briefly, Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd., 927 F.2d 1200, 18 USPQ2d 1016, (CAFC 1991) (hereinafter, Amgen or Amgen v. Chugai) concerns the infringement and validity of two patents, U.S. Patent No. 4,703,008, assigned to Amgen, Inc. and U.S. Patent No. 4,677,195, assigned to Genetics Institute (hereinafter, the '008 and '195 patents, respectively). One of the court's considerations in this case was the validity of claims 2 and 7 of the '008. Claim 2 was challenged as obvious in view of the prior art and was held valid by the court. Claim 7 was challenged as lacking enablement under 35 U.S.C. §112 and was held to be invalid by the court.

The claims at issue here are very different from the claim in the Amgen patent to which the Examiner refers. Claim 7 in U.S. Patent No. 4,703,008 reads:

> 7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake.

Claim 7, thus, reads, not only on peptides encoding erythropoietin but also on other peptides, erythropoietin analogs, i.e. peptides with "EPO-like" activity (see, Amgen Inc. v. Chugai Pharmaceutical Co. Ltd. at 1028), that have a similar sequence such that they possess two biological properties in common with erythropoietin. The Court found that the claims encompass analogs of erythropoietin and that Amgen had failed to find any erythropoietin analogs that possess both requisite biological properties. In addition, the supporting language in the specification defined the DNA fragments to include any that hybridize; the language of the instant claims that is more limited and is not intended to encompass alpha2-like-, alpha3-like- and beta2-like-encoding DNA.

Thus, the claims are far more narrowly drawn than those at issue in Amgen Inc. v. Chugai Pharmaceutical Co. Ltd. and are more analogous to claim 2 of Amgen, which has been held valid. Claim 2 recites:

> A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding erythropoietin.

In this instance, most of the claims at issue herein are more limited than claim 2 of Amgen, since the instant claims include sequence limitations and functional limitations and the other claims are of similar scope to claim 2 of Amgen. In addition, the instant specification provides for definitions of the intended scope of the claims. Also, claim 7 of patent at issue in Amgen defined the encoded DNA with reference to the biological activity of the encoded protein; the specified biological activities do not necessarily uniquely define erythropoietin. Thus, the claim was held to encompass erythropoietin analogs.

With respect to invalidated claim 7, the court held that "it (was) not sufficient, having made the gene and a handful of analogs whose activity (had) not been clearly ascertained, to claim all possible genetic sequences that have EPO-like activity," Amgen v. Chugai, 18 USPQ2d 1016 at 1028, emphasis added. The court also states, however, that "[i]t is well established that a patent applicant is entitled to claim his invention generically, when he describes it sufficiently to meet the requirements of Section 112," directing attention to Utter v. Hiraga, 845 F.2d 993, 998, 6 USPQ2d 1709, 1714 (Fed. Cir. 1988); and In re Robins, 429 F.2d 452, 456-457, 166 USPQ 552, 555 (CCPA 1970). Thus, claim 2 of the '008 patent, directed to "DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin," was properly enabled by the specification.

Furthermore, the specification defines all terms used in the claims and clearly sets forth the intended scope contemplated by each term. As established below, the claims do not encompass DNA encoding any and all such receptors but a clearly defined and readily obtainable subset thereof. Therefore,

the specification is not comparable to that in U.S. Patent No. 4,703,008, in defining the intended scope of claim 7.

As noted above, the instant claims are more narrowly drawn than that at issue in Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.. First, the instant claims are limited to DNA encoding the **human** neuronal NAChR $\alpha_2$, $\alpha_3$ or $\beta_2$ subunits and to cells containing the DNA. Thus, the claims are limited to DNA encoding particular subtypes of each receptor subunit. The DNA includes DNA having substantially the same sequence as the deposited DNA and DNA that encodes subunits that have substantially the same sequence as the subunits encoded by the deposited plasmids or that encode proteins that include the sequences of amino acids set forth in the figures.

**B. It would not require undue experimentation to make and use the claimed DNA fragments and cells**

As stated above, the inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

As discussed below the claims are commensurate in scope with the disclosure, which exemplifies particular embodiments within the scope of the claims and also teaches how one of skill can obtain other embodiments within the scope of the claims.

**The level of skill in the art is high**

The level of skill in this art is recognized to be high (see, e.g., Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). In addition, the numerous articles and patents that are of record in this application that are authored by those of a high level of skill for an audience of a high level of skill further evidences the high level of skill in this art.

**The scope of the claims**

The claims encompass DNA that encodes the subunits encoded by the deposited clones or that have only minor sequence variations (or degenerate codons). The claims are limited to the specified subtypes and to minor variants thereof that fall within the definitions provided in the specification. In light of the limiting definition of each subunit, the functional language in the claims limiting each claim to DNA encoding each subunit or cells containing the DNA encoding the specified human neuronal nicotinic acetylcholine receptor subunits, the description of sequences of each subunit and the deposit of clones containing DNA encoding each subunit, as well as the high level of skill in the art. Only those DNA fragments that have are the same or have minor, inconsequential variations of the DNA fragments disclosed in the specification, such as degenerate codons, are encompassed by the instant claims.

Claims are intended to be read in light of the specification, thus the definition of substantial sequence homology, which refers to minor and inconsequential sequence variations, cannot be ignored. The claims simply do not encompass DNA encoding analogs of each subunit, but are intended to encompass the disclosed subunits or minor variants thereof.

The claims are limited to specific subtypes of $\alpha$ and $\beta$ subunits. As disclosed in the specification, the sequences of $\alpha_3$ and $\alpha_2$ are homologous, but distinct subunits. So that, in reality the variability of sequence that is implicitly in the instant claims is very limited. If DNA is isolated that encodes an $\alpha$ or a $\beta$ subunit that is different to any extent from the sequences disclosed in the

application or the sequences of the deposited clone, such DNA encodes a distinct subunit and, thus, is not within the scope of the instant claims.

Therefore, the claims do not encompass innumerable fragments, insertions and rearrangements that read on unrelated proteins. The claims include two substantive limitations: 1) the DNA encodes either a human neuronal nicotinic acetylcholine receptor $\alpha_2$, $\alpha_3$ or $\beta_2$ subunit **and** 2) is substantially the same as or encodes the same amino acids as the sequence of the DNA set forth in the figures or deposited at the ATCC. Any DNA fragment that encodes an unrelated protein is not encompassed within the instant claims. Any DNA fragment that encodes even a different subtype of the same subunit, such as a $\beta_3$ subunit, is not encompassed within the claims [other than claim 53]. Further, unlike the claim at issue in Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., the claims do not encompass analogs of the subunits. Thus, the claims encompass the specifically disclosed DNA, the deposited DNA and DNA that can be constructed by substituting degenerate codons for the codons in the disclosed or deposited DNA, and DNA that encodes minor, but equivalent, amino acid alternations, such as DNA that encodes a protein that includes a single conservative amino acid change.

In addition, there is guidance presented in the specification for isolating DNA, there are deposited clones that comprise DNA that encodes all or a substantial portion (5 nucleotides are missing from the deposited $\alpha_2$-encoding clone) of each subunit. Also, partial or complete sequences of each subunit are set forth in the specification.

**Teaching and guidance in the specification**

The specification teaches how to introduce the DNA encoding the subunits into host cells, express such DNA to produce functional heterologous nicotinic acetylcholine receptors and test such for activity. Thus, the specification provides sufficient guidance to permit those of skill in the art to

ascertain whether a particular subunit encoded by DNA that is not identical to the deposited or disclosed DNA encodes a functional subunit.

Patents are written to enable those of skill in the art to practice the invention. A patent need not disclose what is well known in the art (W.L. Gore & Assoc. v. Gorlock, Inc., 721 F.2d 1540, 1556, 220 USPQ 303, 315). In this instance, those of skill in the art would have access to the deposited DNA and would be able, if necessary, to sequence the DNA encoding each subunit and/or to use the deposited clones to readily isolate other such clones.

The specification defines (page 11, lines 7-31) what is encompassed by the DNA encoding each subunit and defines substantial sequence homology. For example, the specification states that $\alpha_2$ "refers to a gene, which has been identified in chick and rat, that encodes a neuronal subunit of the same name". In addition, the specification provides a partial DNA sequence of the $\alpha_2$ subunit, a restriction map of the clone, and indicates that a plasmid encoding the $\alpha_2$ has been deposited in a recognized depository. The $\alpha_3$ and $\beta_2$ subunits are similarly defined. Further, the specification teaches that the both subunits are required for formation of a functional ligand-gated channel and defines substantial sequence homology to encompass only minor and inconsequential sequence variations. The specification teaches DNA encoding each of the subunits and provides deposits that contain this DNA and also, as discussed above, defines what is intended for each DNA clone encoding the each subunit to encompass.

One of skill in this art could readily obtain the deposited clones or obtain DNA having the sequence set forth in the figures and one of skill in this art could readily substitute degenerate codons for the codons set forth in the figures or the codons in the deposited DNA. The DNA can be identified by comparison with the sequence of the disclosed or deposited DNA and by inclusion of an open reading frame that encodes one of the specified subunits.

**Conclusion**

Therefore, it would not require undue experimentation for one of skill in the art to make and use the claimed subject matter. Therefore in light of the instant specification, no undue experimentation is required to isolate DNA encoding the $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits.

Furthermore, it is unfair and unduly limiting to require applicant to limit the claims to only the disclosed embodiment. To do so is contrary to the public policy upon which the U.S. patent laws are based. If applicant is required to limit the claims to only the DNA contained in the deposited plasmids, then those of skill in the art could by virtue of these deposits isolate DNA encoding closely related subunits or readily modify the disclosed DNA and practice what is disclosed in the application, but avoid infringing such limited claims. To permit that is simply not fair. The instant application teaches DNA encoding each of these subunits and thereby provides a means for others to isolate such subunits. As is apparent from the specification and the DECLARATION, this was clearly not a routine task, but required creative invention to have obtained the clones. Thereafter, having such clones, permits one of skill to readily isolate related clones. Thus, the disclosure and the deposits permit others to readily clone other such subunits or to make minor changes in deposited DNA and thereby avoid infringement.

Certainly, applicant is entitled to claims to the deposited plasmids and/or to the portion of each plasmid that comprises DNA that encodes all or a portion of a nicotinic acetylcholine receptor subunit. Applicant is the first to have isolated any human nicotinic acetylcholine receptor-encoding DNA, and as such should be entitled to claims directed thereto.

**C. The Examiner contends that conception of scope beyond the deposited clones has not occurred**

It is respectfully submitted that (a) the holding in Amgen is misapplied; (b) the instant claims do not read on any and all human neuronal nicotinic acetylcholine receptor subunits; and (c) the instant specification teaches how to make and use the claimed DNA fragments and cells without undue experimentation (this is discussed below with respect to the scope of enablement rejection.

**(a)** The holding in Amgen

It is respectfully submitted that Amgen does not stand for the proposition that the sequence of every DNA molecule within the scope of a claim must be known. The portion of Amgen relied on by the Examiner arose in the context of an interference in which the date of invention of the DNA encoding erythropoietin was determined. It is addressing the issue of when conception of the gene occurred; it does not address scope of enablement. The passage does not address the issue of adequate enablement of patent specifications and claims. The court states, in that passage, that:

> when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, *i.e.*, until after the gene has been isolated.

Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd., 18 U.S.P.Q. 2d, 1016.

Amgen holds that conception of a gene occurs when one can distinguish it from among others and describe how to obtain it. It had been argued in Amgen that preparation of probes and a method of use of the probes for isolation of erythropoietin-encoding DNA constituted conception. The court stated that until one had done the experiment, i.e., actually used the probes and the method and isolated a DNA clone, conception had not occurred.

This holding in Amgen does not resolve or address the issue of what scope one is entitled to having cloned a DNA molecule that encodes a particular

gene. This portion of the Amgen case is only relevant to the issue of conception, i.e., when the first gene was obtained. Scope of enablement is not addressed in this context.

Thus, Amgen does not hold that one cannot obtain protection for more than one DNA molecule based on a disclosure of one molecule. In fact, as discussed below, a claim, claim 2 in U.S. Patent No. 4,703,008, directed to "DNA encoding erythropoietin" was deemed valid.

In discussing scope of enablement, the court states that for claims to DNA molecules an enabling disclosure requires teaching "how to make and use enough sequences to justify grant of the claims sought" [see, page 1027, col. 1]. In discussing claim 7 of U.S. Patent No. 4,703,008 , which read:

> 7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake.

the Court indicted that the disclosure in the patent "might well justify a generic claim encompassing erythropoietin and similar analogs", but it is inadequate support for claims to erythropoietin-like molecules (claim 7) that have a specified activity and are have a sequence that is sufficiently duplicative to possess the specified activities. In contrast, the court held that claim 2 (directed to DNA encoding erythropoietin) is valid. Such claim does not specify the sequence of the DNA.

The instant application does provide sufficient disclosure to constitute "conception" of DNA encoding neuronal nicotinic acetylcholine receptor subunits because it defines each subunit subtype, defines it so as to distinguish it from other materials and describes how to obtain it. The specification provides a description of the structure of DNA fragments encoding several

neuronal nicotinic acetylcholine receptor subunits, how to obtain them, and how to use them to obtain other such DNA fragments and full-length clones.

**THE REJECTIONS OF CLAIMS 53, 54, 57-63, 66-68, 70 and 72-75 UNDER 35 U.S.C. §103**

Prior to addressing the specifics of the rejections in this application, it is noted that this application is a continuation-in-part of U.S. application Serial No. 07/504,455, which is now U.S. Patent No. 5,369,028. This patent includes claims directed to DNA encoding $\alpha_3$ and $\beta_2$ neuronal nicotinic acetylcholine receptor subunits and cells containing such DNA. In addition, during prosecution of the parent application, claims to DNA encoding $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits were deemed to be free of the prior art. Thus, it is unclear on what basis rejections over Boulter et al. can be maintained in this application.

The DECLARATION of ELLIS that is of record in the parent application and that demonstrated that the prior art references regarding rat nicotinic acetylcholine receptor subunit-encoding DNA do not provide sufficient guidance to permit isolation of human neuronal nicotinic acetylcholine receptor subunit-encoding DNA. It is noted that the Office is required to give full faith and credit to its own determinations. It is also noted that claims 55 and 56 are not within the purview of this rejection. In addition, new claims 82-84 are also not within the purview of this rejection (nor within the purview of any rejection of record).

**Claims 53, 54, 57-63, 66-68, 70 and 72-75**

Claims 53, 54, 57-63, 66-68, 70 and 72-75 are rejected under 35 U.S.C. §103 as being unpatentable over Boulter et al. (1987) Proc. Natl. Acad. Sci. U.S.A. 84:7763-7767, Schofield, et al. (1989) FEBS Letters 244:361-364 (1989), Grenningloh, et al. (1990) EMBO J. 9:771-776 and Noda et al. (1983) Nature 303:818-823 because Boulter et al. teaches DNA of rat origin that encode $\alpha_3$, $\alpha_4$ and $\beta_2$ rat neuronal nicotinic acetylcholine receptor subunits, but not human nicotinic acetylcholine receptors; and Schofield, et al., Grenningloh et al. and Noda et al. allegedly show that isolation of DNA encoding a human

ligand-gated ion channel subunit from an appropriate library using a probe from another mammal was routine, and that genes among this superfamily were known to be highly conserved, thereby indicating that one of ordinary skill in this art would have had a reasonable expectation of success.

It is concluded that because:

> an artisan of molecular biology knew that the value of neuroreceptor research such as that described in the Boulter et al. publication would ultimately lie in its applicability to human subjects, the artisan would have found it obvious to have isolated nucleic acids encoding human homologs of the rat nicotinic acetylcholine receptor subunits by probing a human neuronal library with nucleic acid probes encoding rat subunits using those methods described in each of Grenningloh et al., Schofield et al., and Noda et al.

This rejection is respectfully traversed.

It is respectfully submitted that this rejection is defective in at least three aspects: (1) none of the cited references teaches or suggests that subunits homologous to the rat subunits exist in humans; (2) none of the cited references teaches or suggests anything regarding rat $\alpha_2$ subunit; (3) as demonstrated in the DECLARATION and discussed below the references fail to teach appropriate library; and (4) as demonstrated in the DECLARATION, the cited references do not provide a reasonable expectation of success. As described in the DECLARATION, following the teachings of the references does not necessarily produce expected results nor, in the case of $\alpha_4$, permit successful isolation of DNA encoding subunits that correspond to the rat subunits.

**Relevant law**

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must

actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Also of interest with respect to the issue of obviousness in this case is the holding in In re Deuel in which the court held:

> We today reaffirm the principle, stated in Bell, that the existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious, in the absence of other prior art that suggests the claimed DNAs. A prior art disclosure of a process reciting a particular compound or obvious variant thereof as a product of the process is, of course, another matter, raising issues of anticipation under 35 U.S.C. §102 as well as obviousness under § 103. Moreover, where there is prior art that suggests a claimed compound, the existence, or lack thereof, of an enabling process for making that compound is surely a factor in any patentability determination. See In re Brown, 329 F.2d 1006, 141 USPQ 245 (CCPA 1964) (reversing rejection for lack of an enabling method of making the claimed compound). **There must, however, still be prior art that suggests the claimed compound in order for a prima facie case of obviousness to be made out; as we have already indicated, that prior art was lacking here with respect to claims 5 and 7. Thus, even if, as the examiner stated, the existence of general cloning**

> **techniques, coupled with knowledge of a protein's structure, might have provided motivation to prepare a cDNA or made it obvious to prepare a cDNA, that does not necessarily make obvious a particular claimed cDNA.** "Obvious to try" has long been held not to constitute obviousness. In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1680-81 (Fed. Cir. 1988). A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out. Thus, Maniatis's teachings, even in combination with Bohlen, fail to suggest the claimed invention.

A critical inquiry in determining obviousness is whether there would there have been a reasonable expectation of success in achieving the desired goal, applying only the knowledge evidenced as being part of the prior art. In re O'Farrell, 853 F. 2d 894, 903, 7 USPQ 1673, 1681 (Fed. Cir. 1988). Prior art does not render a claim or claims obvious when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the directed result, or that the claimed result would be obtained if certain directions were pursued. The claimed invention is not obvious if the prior art provides no indication of which parameters are critical or provides no direction as to which choices are likely to be successful so that success must be achieved by varying all parameters or trying each of numerous possible choices until one possibly arrives at a successful result. Further, the claimed invention is not obvious if the prior art gives only general guidance on the form of a particular invention or how to achieve it. In O'Farrell the court states:

> [o]bviousness does not require absolute predictability of success. Indeed for many inventions that seem quite obvious, there is no absolute predictability of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious ((In re Merck & Co., 800 F.2d 1091, 1098, 231 USPQ 375, 380 (Fed. Cir. 1986); Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d

1452, 1461 221 USPQ 481, 488 (Fed. Cir. 1984); In re Papesch, 315 F.2d 381, 386-87 137 USPQ 43, 47-48 (CCPA 1963).

In In re O'Farrell, the claims at issue were rejected over two of the inventor's own publications that contained a detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art and evidence suggesting that such modification would be successful.

In the instant case, however, as discussed below, the reasonable expectation of success present in O'Farrell is absent. In addition, the references cited by the Examiner do not provide an enabling methodology and the combination of references does not teach or suggest the instantly claimed DNA, proteins, cells and methods.

**The claims**

The instant claims are directed to DNA encoding the $\alpha_2$, $\alpha_3$, and $\beta_2$ subunits of human nicotinic acetylcholine receptors, DNA encoding $\alpha$ and $\beta$ subunits having substantial sequence homology with the sequence of nucleotides of the DNA encoding any of the $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits, subunits encoded by the DNA, cells containing the DNA and methods using the cells.

**Teachings of the cited references**

Boulter et al. (1986) teaches DNA encoding a possible neuronal nicotinic acetylcholine receptor $\alpha$-subunit that is homologous to the $\alpha$ subunit of the mouse muscle nicotinic acetylcholine receptor. The DNA was isolated by screening a PC12 cell cDNA library with a 540 base pair fragment from the cDNA encoding the mouse muscle acetylcholine receptor $\alpha$-subunit under conditions of low stringency. Boulter et al. (1987) teaches a family of genes expressed in the rat central and nervous system that encode proteins homologous to the $\alpha$ subunit of the rat muscle nicotinic acetylcholine receptor and that express functional receptors in Xenopus oöcytes that are activated by acetylcholine and nicotine.

In particular, the $\alpha_4$-encoding clone of Boulter et al. was isolated from a hypothalamus library, the $\alpha_3$-encoding clone was isolated from PC12 cells (described in Boulter et al. (1986) Nature 319:368-374, which is of record in this application), and the $\beta_2$ clone was isolated by screening a PC12 library with a partial clone obtained by screening a hypothalamus library with the rat $\alpha_3$ clone.

Boulter does not teach or suggest anything regarding human neuronal nicotinic acetylcholine receptors, nor suggest that subunits homologous to rat subunits exist or that DNA encoding such subunits can be isolated, nor anything regarding the differences or similarities between human and rat neuronal nicotinic acetylcholine receptor subunits or subunit-encoding DNA. In addition, as described in the attached DECLARATION and discussed below, following the teachings of Boulter et al. does not result in the instantly claimed DNA.

**Noda et al.**

Noda et al. teaches isolation of cDNA encoding the $\alpha$-subunit precursor of the calf skeletal muscle acetylcholine receptor. Noda et al. teaches noting regarding the structure of human neuronal nicotinic acetylcholine receptors nor means for isolation of DNA encoding such receptors. Noda et al. suggests that these muscle acetylcholine receptors are conserved among species, but provides no specifics or expectation regarding conserved portions or differences nor any expectations regarding human neuronal nicotinic acetylcholine receptor subunits. Thus, Noda et al. adds nothing to the teachings of Boulter et al. regarding human neuronal nicotinic acetylcholine receptors.

**Schofield et al.**

Schofield et al. describes the isolation of human $GABA_A$ $\alpha 1$ and $\beta 1$ receptor subunit-encoding cDNA clones using bovine $GABA_A$ $\alpha 1$ and $\beta 1$ receptor subunit-encoding cDNA clones to probe a human fetal brain cDNA library. $GABA_A$ receptors belong to the class of receptors for inhibitory neurotransmitters, not to the excitatory neurotransmitters of the glutamate class

of receptors. Schofield also teaches the sequences of cDNA clones and corresponding amino acid sequences of the human $\alpha 1$ and $\beta 1$ subunits, providing the differing bovine amino acids for comparison and determining that the amino acid sequence homology between the human and bovine $\alpha 1$ subunits is 99% and the amino acid sequence homology between the human and bovine $\beta 1$ subunits is 98%.

While stating that the results suggest "that the $GABA_A$ receptor subunits are very highly conserved among the mammalian species," Schofield et al. does not speculate or discuss whether these results may also be applicable to nicotinic acetylcholine receptor subunits. Schofield et al. does not suggest that the techniques used therein may be useful or applicable to the isolation of DNA encoding a human neuronal nicotinic acetylcholine receptors. Schofield et al. does not teach or suggest anything regarding human neuronal nicotinic acetylcholine receptor nor any means for isolating DNA encoding such subunits nor any expectations regarding properties of such subunits.

Thus, Schofield et al. adds nothing to the teachings of Boulter et al. or Noda et al. regarding human neuronal nicotinic acetylcholine receptors.

**Grenningloh et al.**

Grenningloh et al. teaches isolation of cDNA encoding the $\alpha_1$ and $\alpha_2$ variants of the human glycine receptor (hGlyR) using a DNA probe encoding most of the rat GlyR $\alpha$ subunit to screen a human fetal brain cDNA library. Like $GABA_A$ receptors discussed in the Schofield reference and in contrast to nicotinic acetylcholine receptors, the glycine receptors are involved in inhibitory neurotransmission. Significantly, the human GlyR $\alpha_2$ subunit (hGlyR $\alpha_2$), identified and isolated by Grenningloh et al., is a novel variant of the glycine receptor polypeptide. Grenningloh et al. compares the amino acid sequences of the two human GlyR receptor subunits to the rat GlyR subunit, finding that the hGlyR $\alpha_1$ subunit is 99% homologous to the rat GlyR $\alpha_1$ subunit and that the hGlyR $\alpha_2$ subunit is 76% homologous to the same rat $\alpha_1$ subunit. Grenningloh

et al. used a radiolabelled DNA probe encoding most of the rat GlyR $\alpha$ subunit to screen a human fetal brain cDNA library, resulting in isolation of three overlapping cDNA clones covering an open reading frame of 1356 bp. The deduced amino acid sequence of this cDNA was found to be 76% identical to the rat GlyR protein. The same human fetal brain cDNA library was also screened with a 96-fold degenerate $^{32}$P-labelled 23mer oligonucleotide covering possible coding sequences for a contiguous stretch of eight amino acids that are conserved between rat glycine and $GABA_A$ receptor subunits. This resulted in isolation of a cDNA clone with a predicted amino acid sequence sharing 99% homology to the rat GlyR protein. Thus, the first isolated human clone, having 76% homology to the rat GlyR protein was named an $\alpha_2$ glycine receptor subunit and the latter clone, with 99% homology to the rat protein, was dubbed $\alpha_1$ (hGlyR $\alpha_1$ and rGlyR $\alpha_1$ for human and rat $\alpha_1$ subunits, respectively). Significantly, the human GlyR $\alpha_2$ subunit (hGlyR $\alpha_2$), identified and isolated by Grenningloh, was termed a novel variant of the human glycine receptor polypeptide.

Grenningloh et al. also discusses the functional expression of the hGlyR $\alpha_1$ and $\alpha_2$ subunits in human embryonic kidney cell and *Xenopus* oocyte cell systems. The results indicated that the $\alpha_1$ and $\alpha_2$ subunits each produce functional glycine receptors with generally comparable activities to the rat GlyR $\alpha_1$ subunit. Preliminary data is referenced which suggests significant differences, however, between the relative responses of human GlyR $\alpha_1$ and $\alpha_2$ subunits to taurine.

Thus, Grenningloh unsuccessfully attempts to isolate a human GlyR $\alpha_1$ subunit using a cDNA probe constructed from the rat GlyR $\alpha_1$ subunit cDNA. Unexpectedly, Grenningloh isolates a unique human GlyR $\alpha$ subunit variant, $\alpha_2$, by this technique. It is only by use of a 96-fold degenerate oligonucleotide, constructed based on a stretch of eight contiguous amino acids conserved

between rat glycine and $GABA_A$ receptor subunits, that the human $\alpha_1$ GlyR subunit is isolated.

While noting that the subunits of glycine and $GABA_A$ receptors, both of which belong to the class of receptors involved in inhibitory neurotransmission, have been found to share significant sequence similarity and conserved topology with the subunits of nicotinic acetylcholine receptors, Grenningloh does not discuss human nicotinic acetylcholine receptors nor suggest that the techniques used therein to isolate the human glycine receptor subunits may be applicable to isolation of DNA encoding a human nicotinic acetylcholine receptor subunit and does not suggest any suitable methods therefor.

Thus, Noda et al. Grenningloh et al. and Schofield et al. are at most only marginally relevant to the instantly claimed DNA. Schofield et al. is directed to $GABA_A$ receptors and Grenningloh et al. is directed to glycine receptors, and Noda et al. described muscle acetylcholine receptors. None of these references teaches or suggests that human nicotinic acetylcholine receptors subunits homologous to the rat nicotinic acetylcholine receptor subunits exist or that the methods used by Boulter et al. could be in any manner used to obtain DNA encoding human nicotinic acetylcholine receptor-encoding DNA. None of the references, singly or in combination teaches anything about human $\alpha_2$-nicotinic acetylcholine receptors or DNA encoding such receptor.

Thus, none of the cited references teaches or suggests DNA encoding any subunits of human nicotinic acetylcholine receptor subunits nor teaches or suggests anything regarding the relationships of the particular rat subunits to human subunits nor anything regarding a strategy or methodology for cloning DNA encoding the human subunits. None of the references singly or in combination provides an enabling methodology for isolation of DNA encoding human nicotinic acetylcholine receptor subunits. Contrary to the assertion of the Examiner, as described in the attached DECLARATION, isolation of such

DNA was not straightforward nor were the results achieved predictable or expected.

**The Examiner has failed to set forth a prima facie case of obviousness.**

**The combination of teachings of the cited references does not teach or suggest any of the claimed DNA fragments, cells, subunits or methods, and does not provide a reasonable expectation of success of achieving the desired goal of cloning the claimed human neuronal NAChR subunits.**

As discussed in detail below, none of the cited references or applicant's alleged admission teaches or suggests anything regarding the properties of the claimed human neuronal NAChR subunits. In addition, none of the cited references or applicant's alleged admission, singly or in any combination thereof, teaches or suggests an enabling methodology for cloning human neuronal NAChR subunits or DNA encoding such subunits. Finally, as the attached DECLARATION and following discussion demonstrates, there was no reasonable expectation of success that DNA encoding human subunits could have been cloned.

In fact, at the time of filing this application, applicant was unable to successfully clone all of the subunits that applicant attempted to clone and the particular results achieved were not suggested by the cited art. In addition, the cited art provides no indication of which parameters are critical and provides no direction as to which choices are likely to be successful so that the success that was achieved was accomplished by varying all parameters and trying each of numerous possible choices until full-length clones encoding several of the subunits were eventually isolated or constructed.

As described in the instant specification and in the DECLARATION, a significant problem to be solved in order to clone DNA encoding the human neuronal nicotinic acetylcholine receptor subunits was identifying and obtaining suitable source(s) of cDNA to probe for the desired sequences. Human brain

tissue samples must be obtained post-mortem and must be obtained soon after death in order to minimize autolysis and degradation of the nucleic acids.

The claimed DNA fragments were isolated using rat neuronal acetylcholine receptor subunit DNA fragments as probes in numerous human cDNA libraries. In addition to the difficulty in readily obtaining human neural tissue, NACh receptors are not uniformly expressed throughout the brain and are expressed at very low levels. In addition, as described in the DECLARATION, libraries prepared from RNA from brain tissue that would have been expected to contain mRNA encoding a particular subunit, based on the expression of such subunit in corresponding rat brain tissue, did not contain cDNA that hybridized to the corresponding rat probe. Consequently, it was necessary to probe numerous human cDNA libraries, including pre-frontal cortex cDNA, parietal cDNA, temporal cortex cDNA, brain stem cDNA, basal ganglia cDNA, and spinal cord cDNA, to obtain and identify various fragments of DNA encoding the human neuronal subunits. Then, after partial sequencing and restriction mapping of several such fragments, composite DNA sequences for the human neuronal $\alpha_2$, $\alpha_3$ and $\beta_2$ were deduced. Similar strategies, however, failed to yield human clones encoding the $\alpha_4$ and $\alpha_5$ subunits.

Thus, because of the very low concentration of various human neuronal subunits in the human neuronal tissue, the frequently very localized presence of some of the human neuronal subunits in various sources of tissue, the difficulty in obtaining human neural tissue, including brain tissue, with which to work, as well as the high level of care necessary to ensure the presence of intact mRNA in the source human neuronal tissue, there was no reasonable expectation that DNA encoding any or all of the human subunits could have been obtained. Furthermore, after screening numerous libraries, it was only possible to obtain DNA encoding the human $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits.

None of the cited references provide a methodology for identifying the particularly claimed DNA fragments nor for identifying particular libraries to screen to obtain a particular receptor type subunit and the results obtained, upon screening human libraries were unexpected.

**The cited art does not provide an enabling methodology for cloning DNA encoding the human neuronal NAChR $\alpha_2$ subunit**

For example, as discussed in the DECLARATION, based on information regarding the distribution of receptor subtypes in the rat, such as that taught by Wada et al. ((1989) J. Comp. Neurol. 284: 314-335), indicating that $\alpha_4$ is expressed at high levels in the rat thalamus, a human thalamus cDNA library was screened with DNA encoding the rat $\alpha_4$ NAChR subunit as a probe in order to obtain DNA encoding the human $\alpha_4$ subunit. Unexpectedly, however, the hybridizing clones, Th 2.1, Th 2.111 and Th 2.13 (see Fig. 4 of the application) do not encode the human $\alpha_4$ subunit but do encode the $\alpha_2$ subunit. In addition, the regions of clones Th 2.1 and 2.13 that were sequenced are only about 80% homologous to the rat $\alpha_2$ gene and the regions of the Th 2.111 clone that were sequenced were only about 75% homologous the rat $\alpha_2$ gene. Furthermore, as described in the DECLARATION and discussed below, applicant has not as yet successfully isolated DNA encoding a human neuronal NAChR $\alpha_4$ subunit.

**The cited art does not provide an enabling methodology for cloning DNA encoding the human neuronal NAChR $\alpha_3$ subunit**

The full-length human nicotinic acetylcholine receptor $\alpha_3$ subunit was isolated by screening a cDNA library prepared from dibutyryl cyclic AMP-induced IMR32 cells, which are human neuroblastoma peripheral nervous system cells, using rat $\alpha_3$ DNA. The rat $\alpha_3$ encoding DNA, however, had been isolated from PC12 cells as taught, for example, by Boulter et al. (1987) and Boulter et al. (1986). PC12 cells exhibit traits more characteristic of CNS cells than peripheral nervous system cells. Furthermore, as taught by Goldman et al.

(1987) Cell 48:965-973, which is of record in this application, the rat $\alpha_3$ subunit appears to be a CNS-associated subunit.

Absent the instant application, one of ordinary skill in the art would not have had a reasonable expectation of successfully cloning the $\alpha_3$ subunit from a library derived from peripheral nervous system tissue. Furthermore, IMR32 cells express few, if any functional nicotinic acetylcholine receptors (see, e.g., Gotti et al. ((1986) Biochem. Biophys. Res. Commun. 137: 1141-1147, and Clementi et al. (1986) J. Neurochem. 47: 291-297, which is of record in this application). As described in the DECLARATION, however, twenty-four clones, including a full-length clone, were isolated from the IMR32 cell library. Thus, the mRNA encoding the $\alpha_3$ subunit appears to be quite abundant in IMR32 cells, even though it has been reported that receptors are rare.

Thus, in view of the failure of the prior art to teach a source of $\alpha_3$-encoding cDNA or to provide insights regarding the structure of the human gene, one of ordinary skill in the art would not have had a reasonable expectation of success that DNA encoding the human $\alpha_3$ subunit could have been obtained.

**The cited art does not provide an enabling methodology for cloning DNA encoding the human neuronal NAChR $\beta_2$ subunit**

As taught by the prior art, see, e.g., Deneris et al. (1988) Neuron 1:45-54, which will be provided in connection with a supplemental IDS submitted under separate cover, a functional channel is formed from $\alpha$ and $\beta$ subunits. As described in the DECLARATION, IMR32 libraries, which contain high levels of $\alpha$-encoding DNA contain very low levels of $\beta_2$ encoding DNA. Thus, in light of the cited art, which suggests that $\alpha$ and $\beta$ are co-expressed in rat tissues, one of ordinary skill in the art would have expected that, if abundant RNA encoding an $\alpha$ subunit is present in a particular cell type, that abundant $\beta$-encoding RNA would also be present. DNA encoding the $\beta_2$ subunit was, however, obtained

from a prefrontal cortex library, despite teachings in the prior art that $\beta$ is not expressed at high levels in the prefrontal cortex.

**The cited art does not provide an enabling methodology for cloning DNA encoding the human neuronal NAChR $\alpha_4$ and $\alpha_5$ subunits**

In light of the same prior art, applicant did not obtain full-length clones encoding the human $\alpha_4$ subunit or the human $\alpha_5$ subunit. As described in the DECLARATION, at least two unsuccessful attempts were made in order to isolate DNA encoding the human $\alpha_4$ subunits. Rat $\alpha_4$ DNA was used to screen a human brain stem cDNA library under low stringency and a habenula cDNA library under conditions of high stringency. Eighteen clones in the brain stem library hybridized to the probe. Some of the hybridizing clones encoded an $\alpha_3$ subunit, but none of the hybridizing clones, however, encoded an $\alpha_4$ subunit. Similarly, none of the hybridizing clones in the habenula library encoded an $\alpha_4$ subunit, although one clone encoded an $\alpha_2$ subunit.

In order to isolate DNA encoding the human $\alpha_5$ subunit, a randomly primed library prepared from RNA isolated from dibutyryl cAMP-induced IMR32 cells was screened under conditions of high stringency with a 1.1 kb fragment including the 5′ end of the coding portion of rat $\alpha_5$-encoding DNA. No hybridizing clones were obtained. Chini et al. (1992) using a probe from the 3′ end of the obtained clones encoding the human $\alpha_5$ subunit. Clearly none of the prior art suggested to applicant an enabling methodology for cloning the $\alpha_5$ subunit.

To the extent that the art does not provide an enabling methodology for cloning DNA encoding subunits that applicant was not successful in cloning, it does not provide an enabling methodology for cloning DNA encoding subunits that applicant successfully cloned. Thus, to the extent the prior art does not provide an enabling methodology for isolation of DNA encoding the $\alpha_4$ and $\alpha_5$ subunits, it does not provide an enabling methodology for isolation of DNA encoding the $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits.

Therefore, merely because the prior art teaches DNA encoding subunits from a related species, rat, does not necessarily lead to the conclusion that one of ordinary skill in this art will have a reasonable expectation of successfully cloning DNA encoding human subunits. None of the cited references teaches or suggests anything regarding the corresponding human subunits. The art does not teach sources of such subunits nor enabling methodology for selecting probes that might be successfully used to screen any library. Only in light of the instant specification, which teaches such clones, would one of ordinary skill in the art have been able to have used the cited art to have obtained DNA encoding the human $\alpha_2$, $\alpha_3$, and $\beta_2$ subunits.

**The fact that the method of isolation may be routine is not a proper basis for rejection of a product**

In addition, in view of the recent CAFC decision In re Deuel, U.S. App. LEXIS 6200 (Fed. Cir. 1995), as well as other cases, including Amgen and In re Bell, it is now clear that rejections based on the method of purification are improper. In Deuel the issue on appeal was:

> whether the combination of a prior art reference teaching a method of gene cloning, together with a reference disclosing a partial amino acid sequence of a protein, may render DNA and cDNA molecules encoding the protein prima facie obvious under §103.

The CAFC held that:

> Because Deuel claims new chemical entities in structural terms, a prima facie case of unpatentability requires that the teachings of the prior art suggest the claimed compounds to a person of ordinary skill in the art. Normally a prima facie case of obviousness is based upon structural similarity, i.e., an established structural relationship between a prior art compound and the claimed compound. . . Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties. Similarly, a known compound may suggest its analogs or

> isomers, either geometric isomers (cis v. trans) or position isomers (e.g., ortho v. para).
>
> In all of these cases, however, the prior art teaches a specific, structurally-definable compound and the question becomes whether the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention. See In re Jones, 958 F.2d 347, 351, 21 USPQ2d 1941, 1944 (Fed. Cir. 1992); In re Dillon, 919 F.2d 688, 692. . . .
>
> The fact that one can conceive a general process in advance for preparing an undefined compound does not mean that a claimed specific compound was precisely envisioned and therefore obvious. A substance may indeed be defined by its process of preparation. That occurs, however, when it has already been prepared by that process and one therefore knows that the result of that process is the stated compound. The process is part of the definition of the compound. But that is not possible in advance, especially when the hypothetical process is only a general one.

Deuel is on point with respect to this rejection which is based on a general process that could possibly have been used to identify (not even isolate) a protein whose existence had not yet been demonstrated. The instantly claimed DNA fragments are previously undefined chemical compounds. The prior art in this instance does not suggest the resulting chemical compounds. As discussed above, prior to isolating the instantly claimed DNA, it was not known nor was it predictable from the cited references what the structure of DNA encoding human nicotinic acetylcholine receptors would be nor what properties of the resulting subunits, whether they are similar or different from rat, would be. In fact, as described in the previous DECLARATION of record and in the specification, some properties are similar and some are different. Such similarities and differences are each indicative of the unobviousness of the DNA.

There is no teaching or suggestion in any of the cited references of modifications of the rat nicotinic acetylcholine receptor-encoding DNA that would have resulted in the instantly claimed DNA. The precise differences are not taught or suggested by any of the cited references.

Following the teachings of the cited references, one of ordinary skill in the art might have suggested that all of the subunits would be 97-99% homologous to rat and all would exhibit similar distributions in the human brain as in the rat brain, but they do not. Thus, the instantly claimed DNA fragments and cells are not prima facie obvious over the cited references.

**Notwithstanding the failure to set forth a prima facie case of obviousness, the combination of teachings of Boulter et al. with those of Schofield et al., Grenningloh et al. and Noda et al. does not teach or suggest the unexpected properties possessed by the subunits encoded by the instantly claimed DNA fragments and cells**

Unexpected properties must always be considered the determination of obviousness (In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963)).

**DECLARATION of JOHNSON**

In the Advisory Action, the Examiner urges that the differences demonstrated between rat nicotinic acetylcholine receptors of the prior art and the human nicotinic acetylcholine receptor cannot be relied upon because these difference were not disclosed in the specification. As stated in the MPEP 716.02(f):

> Advantages not disclosed in appellant's application may not be urged as a basis for the allowance of claims, In re Davies 177 USPQ 381, 385 (CCPA 1973) ("[W]e are of the view that the basic property or utility must be disclosed in order for affidavit evidence of unexpected properties to be offered."; "[T]he public will derive the most benefit from a patent when it discloses on its face those properties or utilitarian advantages which were ultimately persuasive of nonobviousness."), **unless the advantage would inherently flow from what was originally disclosed in the specification.** In re Zenitz, 142 USPQ 158 (CCPA 1964) (evidence that claimed compound minimized side effects of hypotensive activity must be considered because this undisclosed property would inherently flow from disclosed use as tranquilizer); Ex parte Sasajima,

212 USPQ 103 (Bd. App. 1981) (evidence relating to initially undisclosed relative toxicity of claimed pharmaceutical compound must be considered). In re Davies, the court held that the undisclosed properties of toughened polystyrene (i.e., improved gloss, transparency, and processability) would not flow from a disclosure of improved mechanical properties such as impact strength.

In this instance, the results set forth in the DECLARATION of JOHNSON do indeed inherently flow from the specification. The specification teaches that there is a need for human recombinant nicotinic acetylcholine receptors and DNA encoding such receptors for use in drug screening assays in order to identify compounds that are specific for human therapeutic use, not rat therapeutic use. This showing of a difference in pharmacology between rat and human is a result that inherently flows from the specification as originally filed. Applicant is providing this data to demonstrate that, although rat and human receptors share homology, they are have different activities. In particular, they have different activities when used to test compounds for use as nicotine antagonists.

For example, the specification [see, pages 5-6] states:

> **The neuronal clones of the present invention encode a family of acetylcholine receptors having unique pharmacological properties.** The demonstration that the nicotinic acetylcholine receptors are much more diverse than previously expected offers an opportunity for a high level of pharmaceutical intervention and a chance to design new drugs that affect specific receptor subunits. Such subtypes make it possible to observe the effect of a drug substance on a particular receptor subtype, which can be expressed in a recombinant cell in the absence of the other receptor subtypes. Information derived from these observations will allow the development of new drugs that are more specific, and therefore have fewer unwanted side effects.
>
> In addition, the availability of human neuronal receptors makes it possible to perform initial in vitro screening of the drug substance in a test system which is specific for humans. While it is true that the drug eventually has to be administered directly to the human patient, it is probable that useful drugs are being missed because conventional drug screening is limited to assays employing non-human receptors, human tissue preparations (which are likely to be contaminated with other

receptors, both nicotinic and non-nicotinic in origin), and other suboptimal assay systems. Consequently, the ability to screen drug substances in vitro on specific receptor subtype(s) is likely to be more informative than merely screening the drug substance employing presently available suboptimal assay systems.

Both the receptor subunit genes and proteins of the present invention can be used for drug design and screening. For example, the cDNA clones encoding the human alpha2, alpha3 and beta2 receptor subunits can be transcribed in vitro to produce mRNA. This mRNA, either from a single subunit clone or from a combination of clones, can then be injected into oocytes where the mRNA directs the synthesis of the human receptor molecule(s). The resulting receptor-expressing oocytes can then be contacted with a test compound, and the agonist or antagonist effect thereof can then be evaluated by comparing oocyte response relative to positive and negative control compounds and positive and negative control oocytes. Alternatively, the clones may be placed downstream from appropriate gene regulatory elements and inserted into the genome of eukaryotic cells. This will result in transformed cell lines expressing a specific human receptor subtype, or specific combinations of subtypes. The derived cell lines can then be produced in quantity for similar reproducible quantitative analysis of the effects of drugs on receptor function.

Thus, the specification states that the DNA provided in this application encodes receptors or provides means to obtain receptors that have unique pharmacological properties. The DECLARATION of record provides evidence that this is indeed a correct assertion. DNA encoding human nicotinic acetylcholine receptors is obtained in order to develop assays to screen for drugs that are specific for human nicotinic acetylcholine receptors.

Thus, data demonstrating differences between rat and human nicotinic acetylcholine receptors must be considered.
Differences between the human nicotinic acetylcholine receptors and those of the rat could not have been predicted. The previously submitted DECLARATION of JOHNSON demonstrated that human neuronal nicotinic acetylcholine receptors containing $\alpha_3\beta_2$ subunits exhibited different sensitivities to various agonists than the corresponding rat neuronal nicotinic acetylcholine receptors.

Similar experiments using receptors containing $\alpha_2\beta_2$ subunits also showed substantial variation. It is noted, however, that the $\alpha_2$-encoding DNA used in those experiments, were obtained using DNA from the deposited plasmid to obtain DNA encoding a fully functional $\alpha_2$ subunit. The Johnson DECLARATION demonstates a difference in rank order of relative potency among various ligands.

It is also noted that the data are normalized are relative numbers. Thus, any ligand could have been selected and assigned a value of "1" against which all other data is compared.

Therefore, the receptors encoded by the claimed DNA fragments and the cells containing such DNA exhibit properties that are not taught or suggested by the cited references.

**DECLARATION OF ELLIS**

This is substantially an updated (with respect to the present employment of Mr. Ellis) DECLARATION that is of record in the parent application, which is now U.S. Patent No. 5,369,028. This DECLARATION and above discussion demonstrate that there was no reasonable expectation of success that DNA human subunits could have been cloned. It was not possible to successfully clone all of the subunits that applicant attempted to clone and the particular results achieved were not suggested by the cited art. In addition, the cited art provides no indication of which parameters are critical and provides no direction as to which choices are likely to be successful so that the success that was achieved was accomplished by varying all parameters and trying each of numerous possible choices until full-length clones encoding several of the subunits were eventually isolated or constructed. Contrary to the assertion of the Examiner, as described in the attached DECLARATION, isolation of such DNA was not straightforward despite art describing DNA encoding rat nicotinic acetylcholine receptors nor were the results achieved predictable or expected.

As described in the instant specification and in the DECLARATION, a significant problem to be solved in order to clone DNA encoding the human neuronal nicotinic acetylcholine receptor subunits was identifying and obtaining suitable source(s) of DNA to probe for the desired sequences. Human brain tissue samples must be obtained post-mortem and must be obtained soon after death in order to minimize autolysis and degradation of the nucleic acids.

**Conclusions**

Finally, because the prior art teaches DNA encoding subunits from a related species, rat, does not necessarily lead to the conclusion that one of ordinary skill in this art will have a reasonable expectation of successfully cloning DNA encoding human subunits. None of the cited references teaches or suggests anything regarding the corresponding human subunits. The art does not teach sources of such subunits nor enabling methodology for selecting probes that might be successfully used to screen any library. Only in light of the instant specification, which teaches provides such clones, would one of ordinary skill in the art be able to use the cited art to obtain DNA encoding the human $\alpha_2$, $\alpha_3$, and $\beta_2$ subunits.

In addition, the results set forth in the specification and DECLARATION indicate that there are unpredictable differences (which are not a function of the ability and skill of the researcher) between the expression of neuronal nicotinic acetylcholine receptor subunits so that art that only teaches properties of rat clones and rat subunits does not provide sufficient information regarding the corresponding human subunits for one of ordinary skill in this art to have had a reasonable expectation of successfully cloning the human subunits. There are sufficient differences in sequences and subunit distribution in neuronal tissues so that the combination of the cited references would not have produced the instantly claimed nucleic acids and cells.

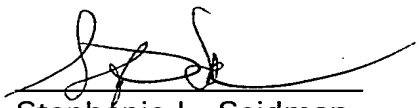
Furthermore, it has been found with respect to other receptors, that small sequence variations have profound effects on the properties of the subunits. Comparisons of properties of AChRs and other receptors among species and in a single species suggest that small amino acid sequence differences produce substantial functional differences. Introduction of three amino acids from a segment of a glycine receptor into the corresponding segment of an $\alpha$ subunit of a chicken nicotinic AChR converts the ion selectivity of the nicotinic receptor from cationic to anionic (see, Galzi et al. (19992) Nature 359:500-505). Studies of AChRs from TE671 cells indicate that, although human muscle AChRs are very similar to muscle AChRs from other species, the pharmacological properties of human muscle AChRs differ from muscle AChRs of other species (see, e.g., Schoepfer et al. (1989) in Molecular Biology of Neuroreceptors and Ion Channels Maelicke, A. (Ed.), NATO-ASI Series, Springer Vergal, Heidelberg). These differences include a lower affinity for the neurotoxin $\alpha$-bugarotoxin ($\alpha$-Bgt) than AChRs from mouse BC3H-1 cells and Torpedo electric organ. Also the two ACh binding sites exhibit comparable sites for curare; whereas, the two ACh binding sites of AChRs in mice and Torpedoes have different affinities. Thus, although the AChRs from Torpedo electric organ or mouse muscle and TE671 AChRs share substantial sequence homology, the AChRs exhibit pharmacological differences. These results and others indicate that the small sequence differences among receptor subunits from different species may be responsible for differences in pharmacology and activity and function among subunits from different species. Thus, the differences in sequences in the human and rat DNA or protein could not have been predicted nor can the DNA encoding the human be merely an obvious variation of the DNA encoding the rat because there is no teaching or suggestion in the cited art regarding the functional significance of each amino acid.

Thus, because of the very low concentration of various human neuronal subunits in the human neuronal tissue, the frequently very localized presence of some of the human neuronal subunits in various sources of tissue, the difficulty in obtaining human neural tissue, including brain tissue, with which to work, as well as the high level of care necessary to ensure the presence of intact mRNA in the source human neuronal tissue, there was no reasonable expectation that DNA encoding any or all of the human subunits could have been obtained. Furthermore, after screening numerous libraries, none of which were suggested by the prior art to be a source of the human DNA, it was only possible to obtain DNA encoding the human $\alpha_2$, $\alpha_3$ and $\beta_2$ subunits.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
BROWN, MARTIN, HALLER & McCLAIN

By: 
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